FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

HOST-PATHOGEN INTERACTIONS

Characterizing a Chimera: Comparative Analysis of Pal Endolysin and its Homologs

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Endolysins - Background

- **Endolysins** are proteins produced by bacteriophage that break down the peptidoglycan cell walls of host bacteria, resulting in cell lysis and the release of progeny virions (Oliviera et al., 2013)
- Bacteria produce similar proteins called **autolysins** that are important in cell division (Cullin et al., 2017)
- Many endolysins have a two domain structure with an **enzymatically active domain (EAD)** that cleaves bonds and a **cell wall binding domain (CBD)** that anchors the endolysin to the cell wall and determines specificity (Linden et al., 2021)
- Researchers can combine EADs and CBDs from different bacteriophage to engineer **chimeric endolysins**, which show great promise for treating antibiotic resistant bacterial infections (Linden et al., 2021)

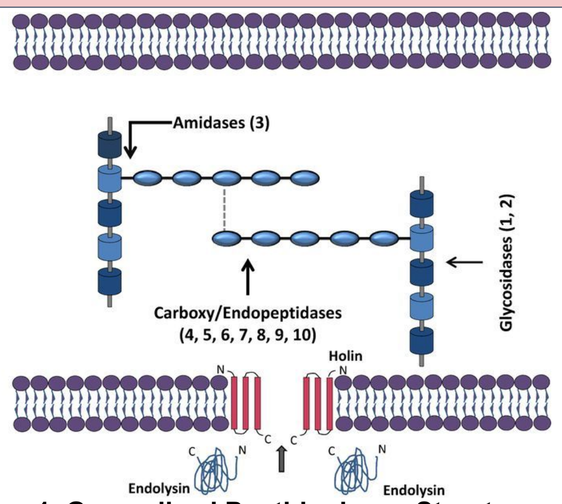


Figure 1. Generalized Peptidoglycan Structure Showing Endolysin Cleavage Sites. Cylinders represent sugars and ovals represent amino acids. Glycosidic bonds link sugars together, peptide bonds link amino acids together, and amide bonds connect sugars to amino acids. Retrieved from Oliviera et al., 2013.

Pal Endolysin

- Produced by Bacteriophage Dp-1, which infects *Streptococcus pneumoniae* bacteria (García et al., 1984)
- Pal's EAD cleaves the bond between **N-acetylmuramic acid** (a sugar) and **L-alanine** (an amino acid) (García et al., 1984)
- Pal's CBD consists of six **choline binding repeats** (CBRs) (Maestro and Sanz, 2016) and ends with a tail that contributes to **dimerization** (Nelson, 2021, personal communication)
- Pal is a **natural chimeric endolysin** (Sheehan et al., 2003)
  - Pal's EAD is homologous to that of Orf259, an amidase produced by Bacteriophage BK5-T, which infects *Lactobacillus lactis*
  - Pal's CBD is homologous to LytA, an autolysin produced by *S. pneumoniae*
- Pal is effective at killing *S. pneumoniae* (including penicillin resistant strains) both *in vitro* and in mice (Loeffler et al., 2001)

Figure 2. Pal's Functional Domains. Blue blocks represent CBRs. Retrieved from Maestro and Sanz, 2016.

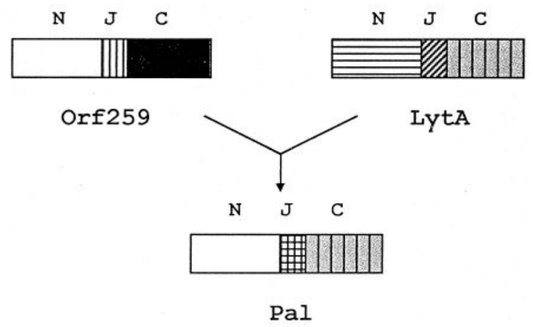


Figure 3. The Chimeric Origins of Pal. N refers to the EAD. C refers to the CBD. J refers to the flexible linker connecting these domains. Retrieved from Sheehan et al., 2003.

Workflow

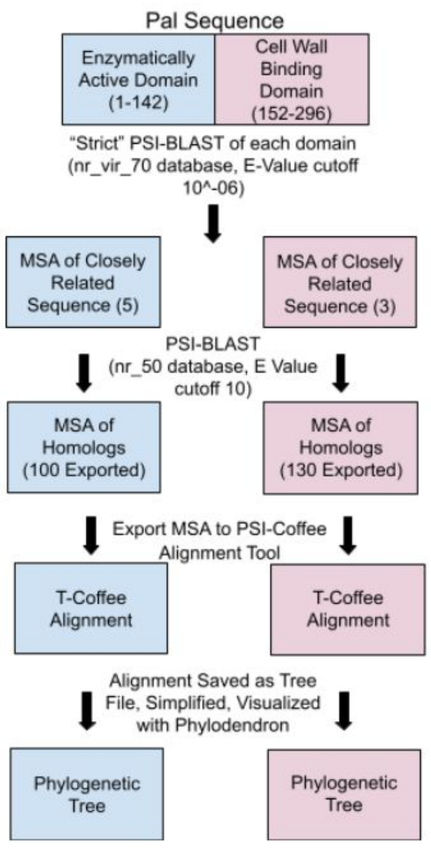
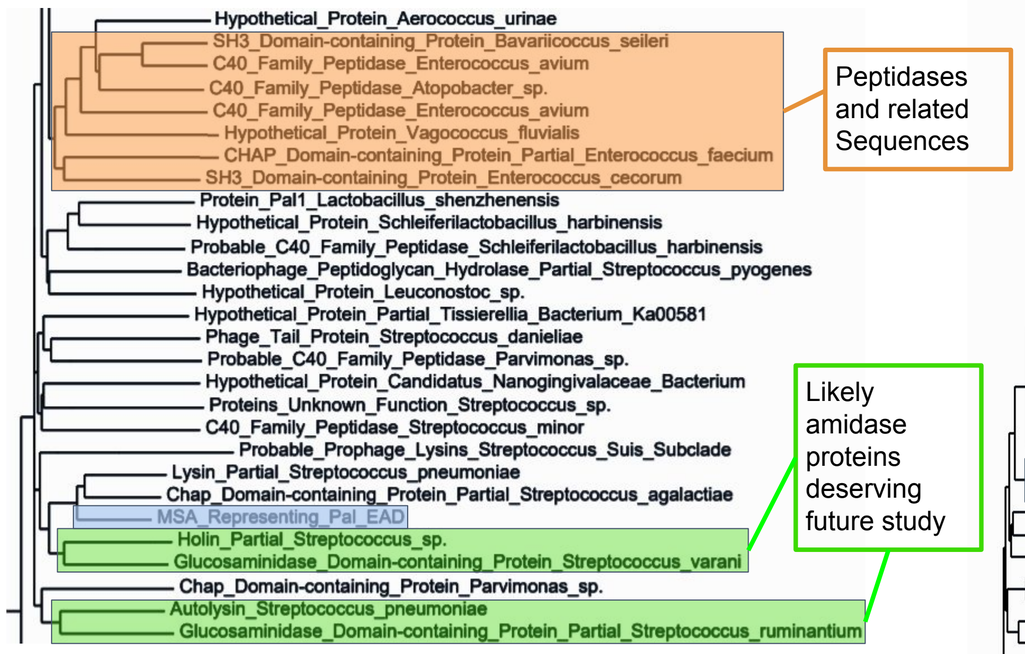


Figure 4. Workflow. To conduct our analysis, we relied on freely available bioinformatics tools from the Max Planck Institute (Gabler et al., 2020). As our inputs were only partial sequences, two iterations were conducted; the first established a small but highly specific MSA of sequences closely related to Pal, while the second searched for homologs. *(Gabler et al., 2020).* This second MSA was exported to T-Coffee (Di Tommaso et al., 2011). Phylodendron visualized the .dnd file generated from T-Coffee.

Results

Phylogenetic Tree of Sequences Homologous to Pal's Enzymatically Active Domain (EAD) [A]



Phylogenetic Tree of Sequences Homologous to Pal's Cell Wall Binding Domain (CBD) [B]

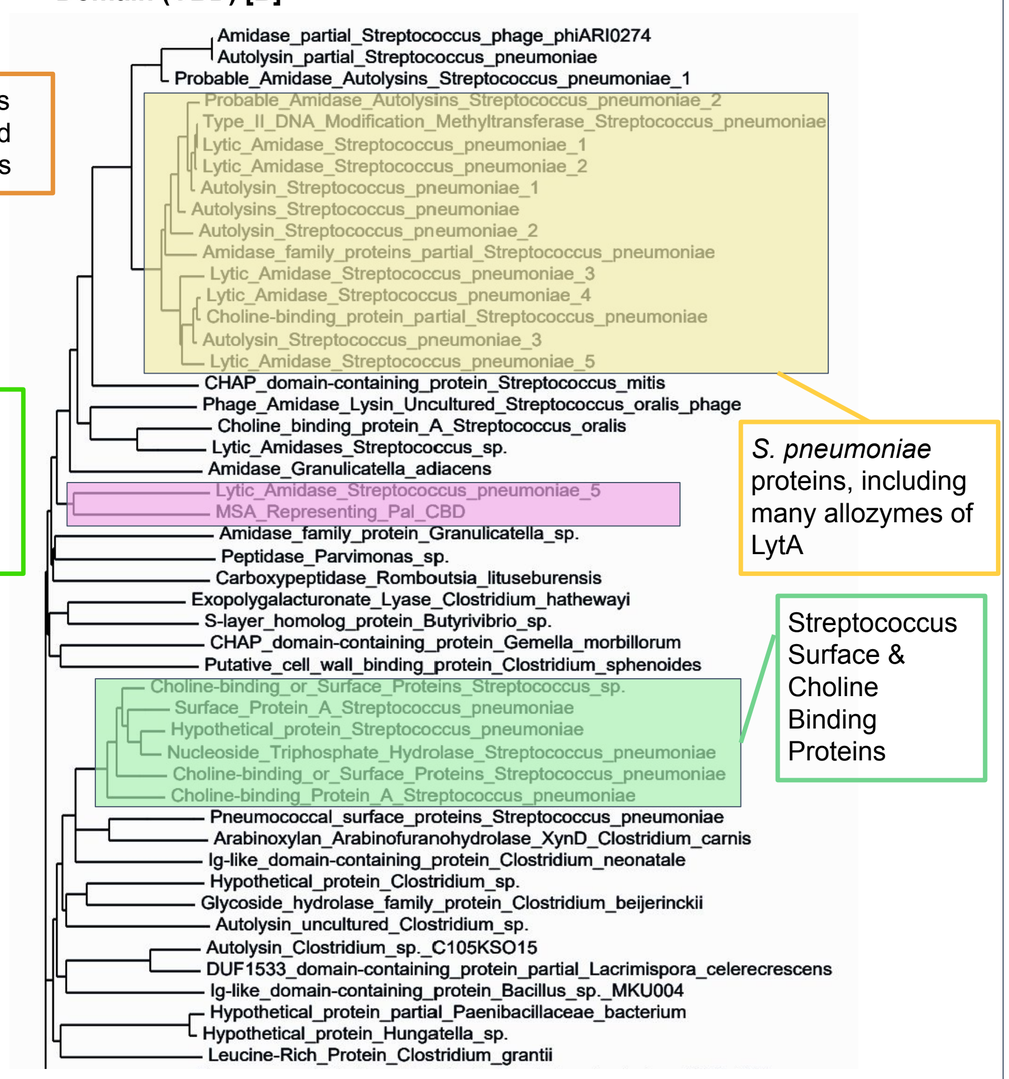


Figure 5. Phylogenetic Trees. A. Pal's EAD is not particularly well conserved. As a result, the homologous proteins on the tree come from a variety of taxonomically distant bacteria and bacteriophage. Many of these proteins are believed to have peptidase activity (orange shaded region). Some of the most closely related sequences appear to be amidases like Pal (green shaded regions). A substantial portion of the proteins are poorly described, hypothetical, or partial, which complicates the phylogenetic relationships further. **B.** The MSA representing Pal's CBD is shown as a sister taxon to a lytic amidase from *S. pneumoniae*, which on further investigation turned out to be an allozyme of LytA, supporting the findings of Sheehan et al. (2003) (pink shaded region). Multiple other allozymes of LytA appear in other locations on the tree (yellow shaded region). In addition to autolysins, many other choline binding proteins and surface proteins from various *Streptococcus* species appear as a clade (green shaded region), suggesting a relatively high degree of sequence conservation.

DISCUSSION and FUTURE DIRECTIONS

- The disparity between the trees for Pal's two domains suggest that these domains have had largely separate evolutionary histories, supporting the idea that Pal is a natural chimera
- Since Pal's EAD and CBD function well together, **it is probable that homologous domains from the other proteins identified on these trees could be combined to produce functional chimeras for therapeutic purposes.**
- The proteins identified in figure 5. A, shaded in green, represent promising candidates for future study. As Pal is itself an amidase, these proteins are likely more closely aligned to Pal's EAD. Additionally, these groupings of sister taxa include proteins that illustrate lysin capabilities; the sister taxa most closely related to the EAD MSA includes a holin protein, which works closely with endolysins (Wang et al., 2000), while the sister taxa next most closely related to the EAD MSA includes an autolysin.
- The proteins identified in figure 5, B, shaded in teal, represent promising candidates for future study as it relates to homologs of Pal's CDB. Cell wall binding relies on the recognition of specific molecules present on the external surface of bacteria; choline is a unique component of *Streptococcus* cell walls (Maestro and Sanz, 2016), so the proteins identified here could be useful to enable chimeric endolysins to target *Streptococcus.*

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Acknowledgments

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